

Appl. No.: 09/930,905

Filed: August 16, 2001

Page 3

Amendments to the Claims:

1. (Currently amended) A method of preparing a chemically modified hemoglobin solution comprising an endogenous antioxidant enzyme, said method comprising:

- a) contacting a stroma free hemoglobin solution with at least one ~~filtration means filter~~, wherein a first ~~filter~~ filtration means retains viral particles and allows passage of a filtrate comprising a hemoglobin polypeptide and an endogenous antioxidant enzyme ~~enzymes~~ and the filtrate is substantially free of viral contamination;
- b) chemically modifying the filtrate with an agent; and,
- c) isolating ~~a composition comprising a~~ the chemically modified hemoglobin solution and the endogenous antioxidant enzyme, wherein at least one of the endogenous antioxidant ~~polypeptide~~ enzymes retains enzymatic activity.

2. (Currently amended) The method of claim 1, wherein at least one of the endogenous antioxidant enzymes retaining enzymatic activity is selected from the group consisting of a superoxide dismutase, a catalase, and a glutathione peroxidase.

3. (Currently amended) The method of claim 1, wherein said first ~~filter~~ filtration means allows the passage of at least 50% of the endogenous antioxidant enzymes present in the stroma free hemoglobin solution.

4. (Currently amended) The method of claim 1, wherein the ~~first filter~~ filtration means comprises an AG-Technology a 500,000 molecular weight cutoff filter.

5. (Currently amended) The method of claim 1, wherein said first ~~filter~~ filtration means reduces the passage of viral particles that are between about 200-25 nm in size.

6. (Currently amended) The method of claim 1, wherein said first ~~filter~~ filtration means reduces the passage of viral particles that are 80-100 nm in size.

Appl. No.: 09/930,905

Filed: August 16, 2001

Page 4

7. (Currently amended) The method of claim 1, wherein said first filter filtration means reduces the passage of viral particles that are between about 80-50 nm in size.

8. (Currently amended) The method of claim 1, where said first filter filtration means reduces the passage of viral particles that are between about 50-25 nm in size.

9. (Currently amended) The method of claim 5, wherein said first filter filtration means reduces the passage of said viral particles by about 3 to about 10 log units.

10. (Currently amended) The method of claim 1, wherein said first filter filtration means produces a filtrate having a viral load reduction of at least 3 log units.

11. (Currently amended) The method of claim 1 further comprising contacting the filtrate with ~~at least one~~ a second filter filtration means wherein said second filter filtration means allows the passage of the hemoglobin polypeptide and the endogenous antioxidant enzymes enzyme and retains virus particles.

12. (Canceled)

13. (Currently amended) The method of claim 1, wherein the ~~modifying~~ agent is a bifunctional modifying agent.

14. (Currently amended) The method of claim 13, wherein said ~~modifying~~ agent is selected from the group consisting of a sebacyl chloride, a glutaraldehyde, a diasprin derivatives, a polyaldehydes, a polyoxyethylene, a dextrans, and an insulin.

15. (Currently amended) The method of claim 13, wherein the ~~modifying~~ agent is a bifunctional polyoxyethylene.

Appl. No.: 09/930,905

Filed: August 16, 2001

Page 5

16. (Currently amended) The method of claim 1, wherein the ~~modifying~~ agent is a mixture of a bifunctional and a monofunctional polyoxyethylene.

17. (Currently amended) The method of claim 15, wherein the chemically modified hemoglobin solution comprising an endogenous antioxidant enzyme is PHP.

18. (Currently amended) The method of claim 1, wherein ~~the chemical modification~~ chemically modifying said filtrate with an agent ~~further~~ comprises deoxygenation and pyridoxalation ~~of the hemoglobin~~.

19. (Currently amended) The method of claim 1, wherein ~~the viral contamination of said isolated~~ chemically modified hemoglobin solution comprising an endogenous antioxidant enzyme comprises a hepatitis A viral titer of less than about 1 TCID₅₀ unit/ml.

20. (Original) The method of claim 1, wherein the chemically modified hemoglobin solution comprises about a 50% to about a 200% increase in endogenous red blood cell antioxidant activity per unit of hemoglobin found in red blood cells.

21. (Currently amended) A method of preparing a chemically modified hemoglobin solution comprising an endogenous antioxidant enzyme, said method consisting of:

- a) contacting a stroma free hemoglobin solution with at least one filter ~~filtration means~~, wherein a first filter ~~filtration means~~ retains viral particles and allows passage of a filtrate comprising a hemoglobin polypeptide and the endogenous antioxidant enzyme ~~enzymes~~ and the filtrate is substantially free of viral contamination;
- b) chemically modifying the filtrate with an agent; and,
- c) ~~isolating a composition comprising~~ isolating ~~the chemically modified hemoglobin solution~~ and the endogenous antioxidant enzyme ~~enzymes~~.

22. (Currently amended) A ~~hemoglobin solution comprising~~ a chemically modified hemoglobin solution comprising and at least one endogenous antioxidant enzyme, wherein said

Appl. No.: 09/930,905

Filed: August 16, 2001

Page 6

chemically modified hemoglobin solution ~~modification~~ comprises ~~attachment of~~ a POE linkage, said endogenous ~~antioxidants~~ antioxidant enzyme ~~retains~~ retain enzymatic activity, and said chemically modified hemoglobin solution is substantially free of a viral contamination.

23. (Currently amended) The chemically modified hemoglobin solution of claim 22, wherein said chemically modified hemoglobin solution is PHP.

24. (Currently amended) The chemically modified hemoglobin solution of claim 22, wherein the viral contamination of a viral particle of less than 70 nm in size is of said solution ~~comprises a viral titer of~~ less than about 1 TCID₅₀ unit/ml.

25. (Currently amended) The chemically modified hemoglobin solution of claim 24, wherein the viral contamination titer of said particles that are a viral particle that is 25-30 nm in size is less than about 1 TCID₅₀ unit/ml.

26. (Currently amended) The chemically modified hemoglobin solution of claim 25, wherein said viral particle is hepatitis A.

27. (Canceled)

28. (Currently amended) The chemically modified hemoglobin solution of claim 27, wherein the viral particle is hepatitis A or hepatitis C.

29. (Currently amended) The chemically modified hemoglobin solution of claim 22, wherein said endogenous antioxidant enzyme is selected from the group consisting of a superoxide dismutase, a catalase, a hemoglobin peroxidase, and a glutathione peroxidase.

30. (Currently amended) The chemically modified hemoglobin solution of claim 22, wherein said solution ~~contains~~ comprises between a 50% to a 200% increase in antioxidant activity per unit of hemoglobin found in red blood cells.

RTA01/2137770v1

Appl. No.: 09/930,905

Filed: August 16, 2001

Page 7

31. (Canceled)

32. (Currently amended) A method of decreasing the level of nitric oxide present in the circulation of a mammal, said method comprising, administering to a mammal in a need thereof a therapeutically effective amount of the chemically modified hemoglobin solution of claim 22 in a pharmaceutically acceptable carrier.

33. (Currently amended) The method of claim 32, wherein said chemically modified hemoglobin solution is administered to a mammal having systemic hypotension.

34. (Currently amended) The method of claim 32, wherein said chemically modified hemoglobin solution is administered to a mammal having septic shock.

35. (Currently amended) A method of treating red blood cell loss, said treatment comprising administering to a mammal in need thereof a therapeutically effective amount of the chemically modified hemoglobin solution of claim 22.